

ATTORNEY DOCKET NO. 9530.3

Application Serial No. 10/049,727

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## IN THE CLAIMS

Please amend the claims as follows. This listing replaces all prior versions.

1. (Currently amended) A method for determining active plasminogen activator inhibitor-Type 1 (PAI-1) in a biological fluid, ~~the method comprising the steps of:~~
  - ~~(i) providing a sample of a biological fluid; and~~
  - ~~(ii) measuring the amount of PAI-1/multimeric vitronectin complex in a sample of the biological fluid the sample to determine active PAI-1 in the sample biological fluid.~~
2. (Currently amended) The method of claim 1, wherein ~~step (ii) measuring the amount of PAI-1/multimeric vitronectin complex in the sample~~ comprises the steps of:
  - (a) contacting the sample either simultaneously or stepwise with a first antibody which binds selectively to PAI-1 and a labelled second antibody which binds selectively to multimeric vitronectin; and
  - (b) determining the second antibody bound to the complex to measure the amount of PAI-1/multimeric vitronectin complex in the sample.
3. (Currently amended) The method of claim 1, wherein ~~step (ii) measuring the amount of PAI-1/multimeric vitronectin complex in the sample~~ comprises the steps of:
  - (a) contacting the sample either simultaneously or stepwise with a first antibody which binds selectively to multimeric vitronectin and a labeled second antibody which binds selectively to PAI-1; and
  - (b) determining the second antibody bound to the complex to measure the amount of PAI-1/multimeric vitronectin complex in the sample.
4. (Currently amended) The method of claim 1, wherein ~~step (ii) measuring the amount of PAI-1/multimeric vitronectin complex in the sample~~ comprises the steps of:
  - (a) contacting the sample either simultaneously or stepwise with a first antibody which binds selectively to PAI-1 and a labeled second antibody which binds selectively to multimeric vitronectin;
  - (b) separating the PAI-1/multimeric vitronectin/first antibody/second antibody complex formed in step (a) from the sample; and
  - (c) determining the second antibody bound to the complex to measure the amount of PAI-1/multimeric vitronectin complex in the sample.
5. (Currently amended) The method of claim 1, wherein ~~step (ii) measuring the amount of PAI-1/multimeric vitronectin complex in the sample~~ comprises the steps of:
  - a) contacting the sample either simultaneously or stepwise with a first antibody which binds selectively to multimeric vitronectin and a labelled second antibody which binds selectively to PAI-1;
  - (b) separating the PAI-1/multimeric vitronectin/first antibody/second antibody complex formed in step (a) from the sample; and

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- (c) determining the second antibody bound to the complex to measure the amount of PAI-1/multimeric vitronectin complex in the sample.
6. (Currently amended) The method of claim 1, wherein ~~step (ii) measuring the amount of PAI-1/multimeric vitronectin complex in the sample~~ comprises the steps of:
- (a) simultaneously contacting the sample with a first antibody which binds selectively to PAI-1, the first antibody being immobilized on a solid support, and with a labelled second antibody which binds selectively to multimeric vitronectin; and
  - (b) determining the second antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in the sample.
7. (Currently amended) The method of claim 1, wherein ~~step (ii) measuring the amount of PAI-1/multimeric vitronectin complex in the sample~~ comprises the steps of:
- (a) contacting the sample with a first antibody which binds selectively to PAI-1, the first antibody being immobilized on a solid support;
  - (b) contacting the solid support with a labelled second antibody which binds selectively to multimeric vitronectin; and
  - (c) determining the second antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in the sample.
8. (Currently amended) The method of claim 1, wherein ~~step (ii) measuring the amount of PAI-1/multimeric vitronectin complex in the sample~~ comprises the steps of:
- (a) simultaneously contacting the sample with a first antibody which binds selectively to multimeric vitronectin, the first antibody being immobilized on a solid support, and with a labelled second antibody which binds selectively to PAI-1; and
  - (b) determining the second antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in the sample.
9. (Currently amended) The method of claim 1, wherein ~~step (ii) measuring the amount of PAI-1/multimeric vitronectin complex in the sample~~ comprises the steps of f:
- (a) contacting the sample with a first antibody which binds selectively to multimeric vitronectin, the first antibody being immobilized on a solid support;
  - (b) contacting the solid support with a labelled second antibody which binds selectively to PAI-1; and
  - (c) determining the second antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in the sample.
10. (Currently amended) The method of claim 1, wherein ~~step (ii) measuring the amount of PAI-1/multimeric vitronectin complex in the sample~~ comprises the steps of:
- (a) contacting the sample with a first antibody which binds selectively to PAI-1, the first antibody being immobilized on a solid support;
  - (b) contacting the solid support with a second antibody which binds selectively to multimeric vitronectin;
  - (c) contacting the solid support with a labelled third antibody which binds selectively to

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- (d) the second antibody; and  
determining the third antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in the sample.
11. (Currently amended) The method of claim 1, wherein ~~step (ii) measuring the amount of PAI-1/multimeric vitronectin complex in the sample~~ comprises the steps of:
- (a) contacting the sample with a first antibody which binds selectively to multimeric vitronectin, the first antibody being immobilized on a solid support;
  - (b) contacting the solid support with a second antibody which binds selectively to PAI-1;
  - (c) contacting the solid support with a labelled third antibody which binds selectively to the second antibody; and
  - (d) determining the third antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in the sample.
12. (Currently amended) The method of claim 1, wherein ~~step (ii) measuring the amount of PAI-1/multimeric vitronectin complex in the sample~~ comprises the steps of:
- (a) contacting the sample, either simultaneously or stepwise, with a first antibody which binds selectively to PAI-1 and to which is attached one member of a capture pair and with a labelled second antibody which binds selectively to multimeric vitronectin to form a mixture;
  - (b) contacting the mixture with a solid support on which is immobilized the other member of the capture pair; and
  - (c) determining the second antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in the sample.
13. (Currently amended) The method of claim 1, wherein ~~step (ii) measuring the amount of PAI-1/multimeric vitronectin complex in the sample~~ comprises the steps of:
- (a) contacting the sample either simultaneously or stepwise, with a first antibody which binds selectively to multimeric vitronectin and to which is attached one member of a capture pair and with a labelled second antibody which binds selectively to PAI-1 to form a mixture;
  - (b) contacting the mixture with a solid support on which is immobilized the other member of the capture pair; and
  - (c) determining the second antibody bound to the solid support to measure the amount of PAI-1/multimeric vitronectin complex in the sample.
14. (Previously presented) The method according to claim 1 wherein the biological fluid is selected from the group consisting of whole blood, plasma, serum, urine, saliva, amniotic fluid, cerebrospinal fluid and a tissue extract.
15. (Previously presented) The method according to claim 1 wherein the biological fluid is

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whole blood, plasma or serum.

16. (Currently amended) The method according to claim ~~13~~, wherein the second antibody is labelled with a directly detectable label.

17. (Currently amended) The method according to claim ~~13~~, wherein the second antibody is labelled with a component of a signal-generating system.

18. (Original) The method of claim 17 wherein the component is an enzyme selected from the group consisting of alkaline phosphatase, amylase, luciferase, catalase, beta-galactosidase, glucose oxidase, glucose-6-phosphate dehydrogenase, hexokinase, horseradish peroxidase, lactamase, urease and malate dehydrogenase.

19. (Currently amended) The method according to claim ~~13~~, wherein the second antibody is labelled with a fluorophore.

20. (Original) The method of claim 19 wherein the fluorophore is selected from the group consisting of a coumarin, a rare earth metal ion, chelate or chelate complex, a fluorescein, rhodamine and a rhodamine derivative.

21. (Currently amended) The method of ~~any of claims 1 to 15~~claim ~~3~~, wherein the second antibody is labeled with a luminescent material.

22. (Original) The method of claim 21 wherein the luminescent material is selected from the group consisting of a cyclic diacyl hydrazide, luminol, isoluminol, an acridinium ester, a pyridopyridazine, a dioxerane, a bioluminescent protein and a luciferase.

23. (Previously presented) The method of claim 1 wherein the second antibody is labelled with a label selected from the group consisting of a metal complex, a stable free radical, a vesicle, a liposome, a colloidal particle, a latex particle, a spin label and biotin/avidin.

24. (Currently amended) The method of ~~any one of claims 6 to 13~~claim ~~6~~, wherein the solid support is selected from the group consisting of an ELISA plate, a polyacrylamide matrix, a polystyrene tube, polystyrene beads, latex particles, paramagnetic particles, acrylic particles and gelatin particles.

25. (Original) A kit for determining active PAI-1 in a biological fluid comprising:  
(a) a first antibody which binds selectively to PAI-1; and  
(b) a labelled second antibody which binds selectively to multimeric vitronectin.

26. (Original) A kit for determining active PAI-1 in a biological fluid comprising:  
(a) a first antibody which binds selectively to multimeric vitronectin; and  
(b) a labelled second antibody which binds selectively to PAI-1.

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27. (Previously presented) The kit of claim 25 wherein said first antibody is immobilized on a solid support.
28. (Previously presented) The kit of claim 25 further comprising a set of calibration standards.
29. (Original) A kit for determining active PAI-1 in a biological fluid comprising:
  - (a) a first antibody which binds selectively to PAI-1;
  - (b) a second antibody which binds selectively to multimeric vitronectin; and
  - (c) a labelled third antibody which binds selectively to said second antibody.
30. (Previously presented) The kit of claim 29 wherein said first antibody is immobilized on a solid support.
31. (Currently amended) The kit of claim 29 further comprising a set of calibration ~~is~~ standards.
32. (Previously presented) The kit of claim 26 wherein said first antibody is immobilized on a solid support.
33. (Previously presented) The kit of claim 26 further comprising a set of calibration standards.